Supplemental Information
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The Base-Pairing RNA Spot 42 Participates in a Multioutput Feedforward Loop to Help Enact Catabolite Repression in *Escherichia coli*

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Table S1. Support for the fourth column of Table 1
Evidence supporting catabolite repression and transcriptional regulation by CRP of Spot 42 targets identified by microarray analysis.

Gene	CRP binding site(s)[a]		Effect of glucose[b]	Evidence for CRP regulation[c]	References for CRP regulation
gltA	-83(1) -42(1) -85(2) -65(2)	CAGTGATCCAGGTCACGA TTGTTACAAACATTACCA GATTAACTTATGTAACAG GTGTGGAAGTATTGACCA	↓	C, E	(Wilde and Guest, 1986)
maeA		–	↓	–	
sthA		–	↓	–	
dppB		–	↓	–	
lldP		–	↓	–	
nanC	-70	TTGTGACACGAATTGCAA	N/A	A, B, C, E	(Condemine et al., 2005)
nanT	-69	TAGTGAAGCAGATCGCAT	N/A	A, B, C, E	(Kalivoda et al., 2003)
srlA	-50	TTGCGATCAAAATAACAC	↓	C, D, E	(Yamada and Saier, 1988; Zheng et al., 2004)
xylF	-189	GTGTGCGCTCGCTCGCAA	↓	C, E	(Song and Park, 1997)
ebgC		–	N/A	F	(Stokes and Hall, 1985)
fucIK	-89 -49	AAGTGATGGTAGTCACAT GTGTGACCGCCGTCATAT	↓	A, B, C, E	(Podolny et al., 1999; Zheng et al., 2004)
galK	-45(1) -50(2)	ATTTATTCCATGTCACAC ATTTATTCCATGTCACAC	↓	A, B, C, E	(Irani et al., 1989; Mandal et al., 1990; Rostoks et al., 2000)
gsp		–	↑	–	
yjiA		–	-	–	
ytfJ		–	↓	–	

[a]Numbering designates the distance upstream of the transcriptional start site measured from the 5′ end of the binding site. If multiple transcriptional start sites have been identified, the number in parentheses designates the transcriptional start site associated with the potential CRP binding site. -, no putative CRP binding site sequences found.

[b]Determined by microarray analysis following growth on different carbon sources (Liu et al., 2005). N/A, signals for *nanC*, *nanT*, and *ebgC* were absent under all conditions tested. Up and down arrows indicate upregulation and downregulation by glucose, respectively.

[c]Abbreviations: A, mapped CRP binding site(s); B, binding activity between CRP and promoter; C, presence of putative CRP binding site(s); D, CRP is required for gene activation; E, gene expression analysis; F, evidence suggests that the *ebgAC* operon and the CRP-regulated *lacYAZ* operon descended from a common ancestral operon.

Table S2. Support for the fifth column of Table 1
Evidence for mRNA binding and regulation by Hfq in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium.

Gene	High score in Hfq co-immunoprecipitation in *E. coli*[a]	Enriched in Hfq co-immunoprecitiation in *E. coli*[b]	Enriched in Hfq co-immunoprecitiation in *S. enterica*[c]	Altered expression in Hfq deletion strain of *S. enterica*[d]
gltA	-	Y	Y	-
maeA		Y	-	Y
sthA		Y	-	Y
dppB	Y[f]	Y[f]	Y[f]	Y[f]
lldP		Y	-	-
nanC		-	NP	NP
nanT		-	Y	-
srlA	-	Y	-	-
xylF		-	NP	NP
ebgC		-	NP	NP
fucI		-	-	-
fucK	-	-	-	-
galK[e]	-	Y	-	-
gsp		Y	-	-
yjiA		Y	-	Y
ytfJ	Y[g]	Y	Y[g]	Y

[a]From *E. coli* sense genome microarray (Zhang et al., 2003). Y, gene with high score; -, gene with low score. Not all Spot 42 targets were evaluated in this study.

[b]From *E. coli* high density tiling array (Zhang, A. and Storz, G.; unpublished results). Y, mRNA enriched in co-immunoprecipication; -, mRNA not enriched in co-immunoprecipitation.

[c]From deep sequencing analysis. (Sittka et al., 2008). Y, mRNA enriched in co-immunoprecipication; -, mRNA not enriched in co-immunoprecipitation; NP, gene not present in the genome of *S. enterica*.

[d]From *Salmonella* microarray (Ansong et al., 2009). Y, altered mRNA levels in Hfq deletion strain; -, similar mRNA levels in Hfq deletion strain; NP, gene not present in the genome of *S. enterica*.

[e]The *galK* mRNA was shown to bind Hfq in vitro (Møller et al., 2002).

[f]The upstream gene *dppA* in the same operon was identified.

[g]The upstream gene *ytfK* encoded on the opposite strand was identified.

A

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spf             1                                    GUAGGGUACAGAGGUAAGAUGUUCUAUCUUUCAG  34
                                                                ||||||  ||:|||||
srlA::lacZ     53 AGGGUCAGUGCUGCAACAUUUUGCUGACGUGGUACUCAUUACCAAAGAUAGUAACAAGAGAGGAAGUC -15

spf-I           1      GUAGACGACAGAGGUAAGA------------------------------UGUUCUAUCUUUCAG  34
                       ||  |||||    ||||:|                                ||||||  ||:|||||
srlA::lacZ     53 AGGGUCAG-UGCUG-CAACAUUUUGCUGACGUGGUACUCAUUACCAAAGAUAGUAACAAGAGAGGAAGUC -15
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B

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spf-I           3 AGACGACAGAGGUAA-----------------------------------------------------GAUGUUCUAUCUUU  31
                  |:|||||||  |:||                                                     ||   ||:|||::|
maeA::lacZ     48 UUUGCUGUCCCCUAUAUUUCGCUUGCGACAAAAAACAAAAACCAAGGUACAGUGAGUGAGAAAAAACUUAUAGGUAGGGA -31
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Figure S1. Predicted Base-Pairing Interactions with Spot 42 Variant I, Related to Figure 2

(A and B) The mutations introduced into Spot 42 variant I (*spf-I*) may allow the formation of novel base-pairing interactions with (A) *srlA::lacZ* and (B) *maeA::lacZ* that would explain the increased repression reported in Figure 2. NUPACK predicted no significant base-pairing interactions between *maeA::lacZ* and Spot 42 (*spf*). Mutations yielding *spf-I* are in green and the portion of each fusion corresponding to *lacZ* is in bold.

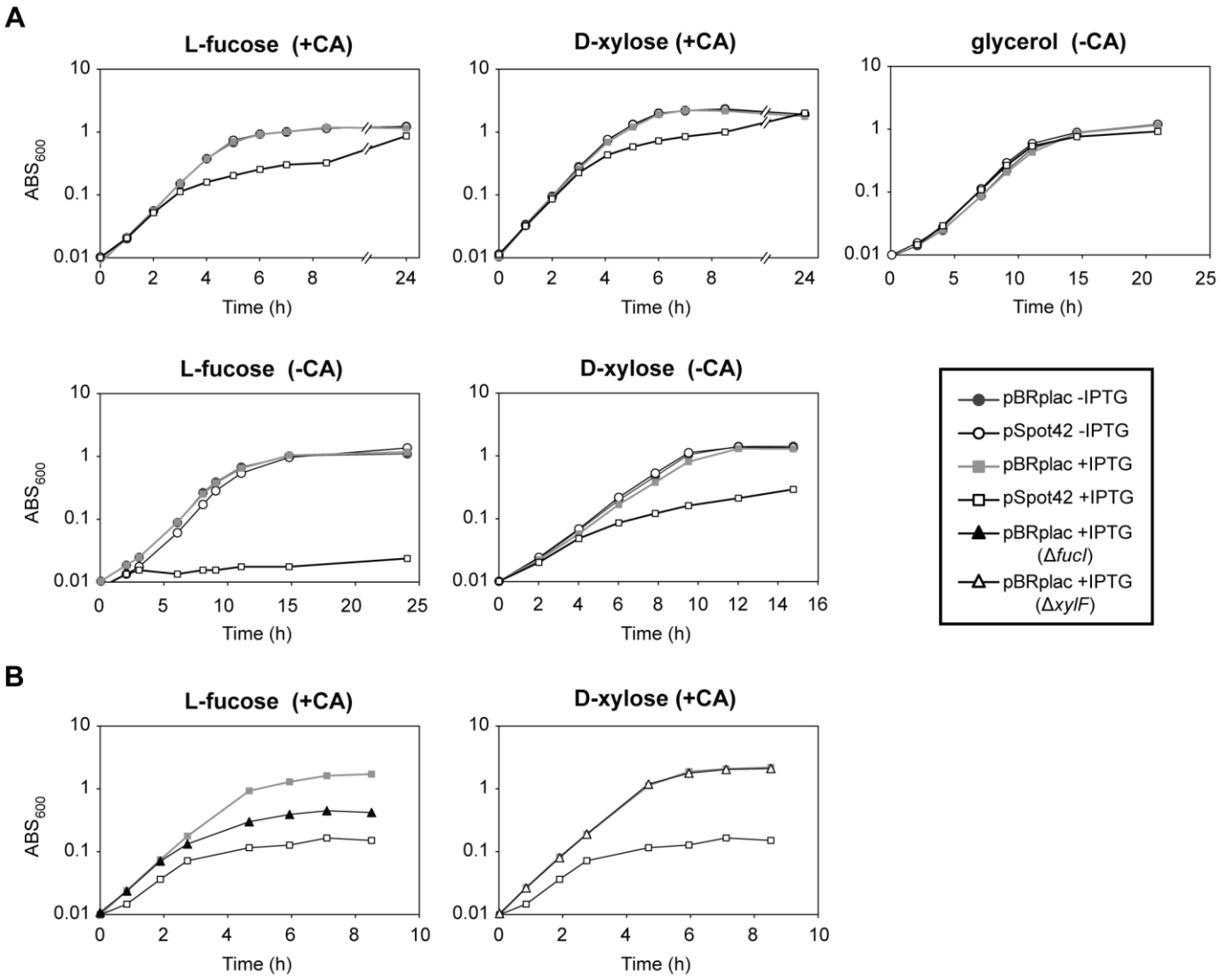


Figure S2. Additional Growth Curves, Related to Figure 4

(A) NM525 Δ*spf::kanR* cells transformed with pBRplac or pSpot42 were grown overnight in M9 containing the indicated carbon source with or without 0.2% casamino acids (CA) and diluted to an OD_{600} of 0.01 into the same type of media with or without 1 mM IPTG. OD_{600} was measured at different times during cell growth. The growth curves for L-fucose (+CA) and D-xylose (+CA) represent the average of measurements from cultures from three separate colonies. Error bars reflecting the standard deviation are smaller than the symbols.

(B) Growth curves for Δ*spf::kanR* Δ*fucI* and Δ*spf::kanR* Δ*xylF* cells. Strains were grown overnight in casamino acid-enriched M9 containing glycerol and 1 mM IPTG and diluted to an OD_{600} of 0.01 into casamino acid-enriched M9 containing the indicated carbon source and 1 mM IPTG. Applied concentrations of specific carbon sources: 0.2% L-fucose, 0.2% D-xylose, 0.4%, glycerol.

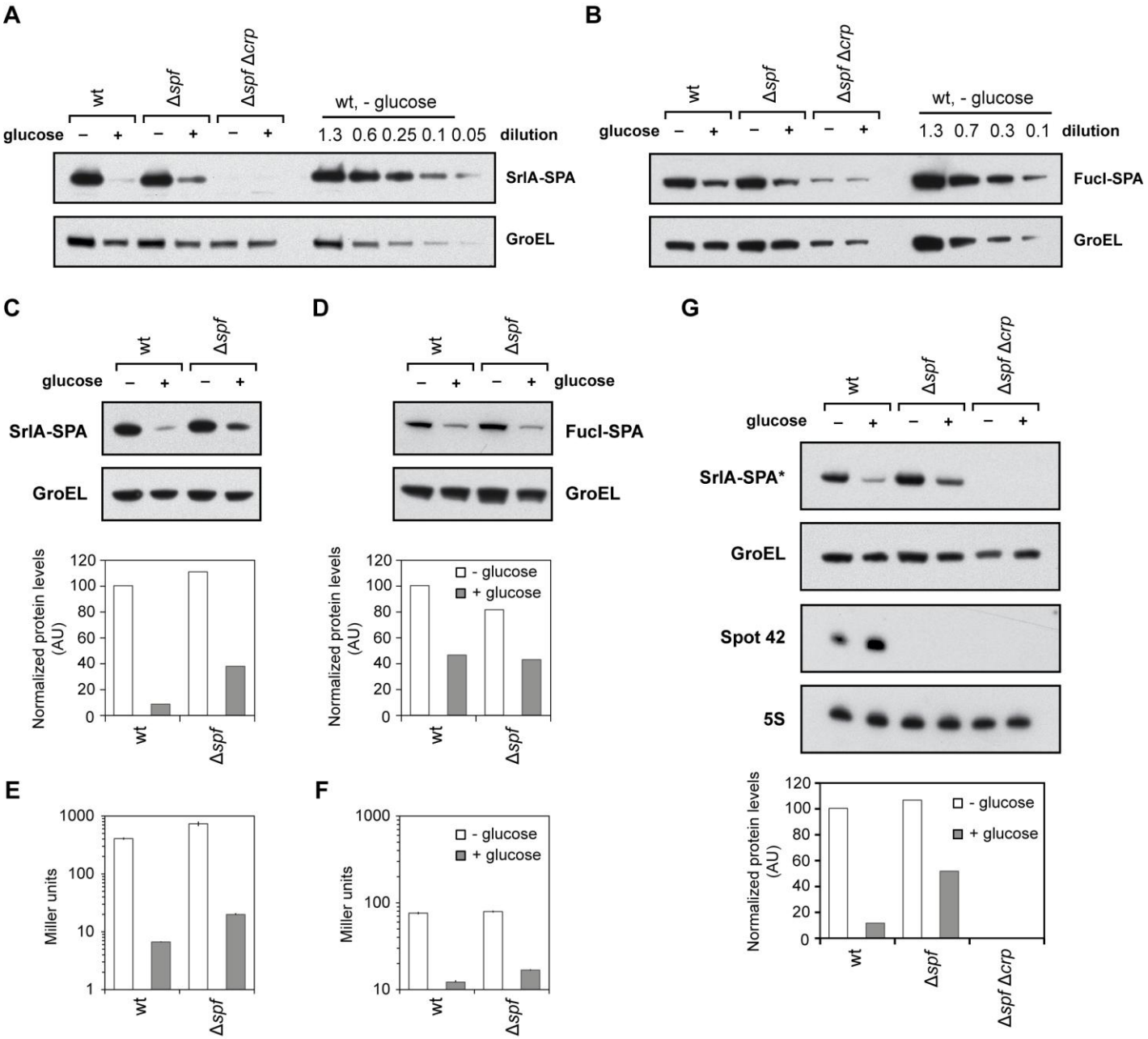
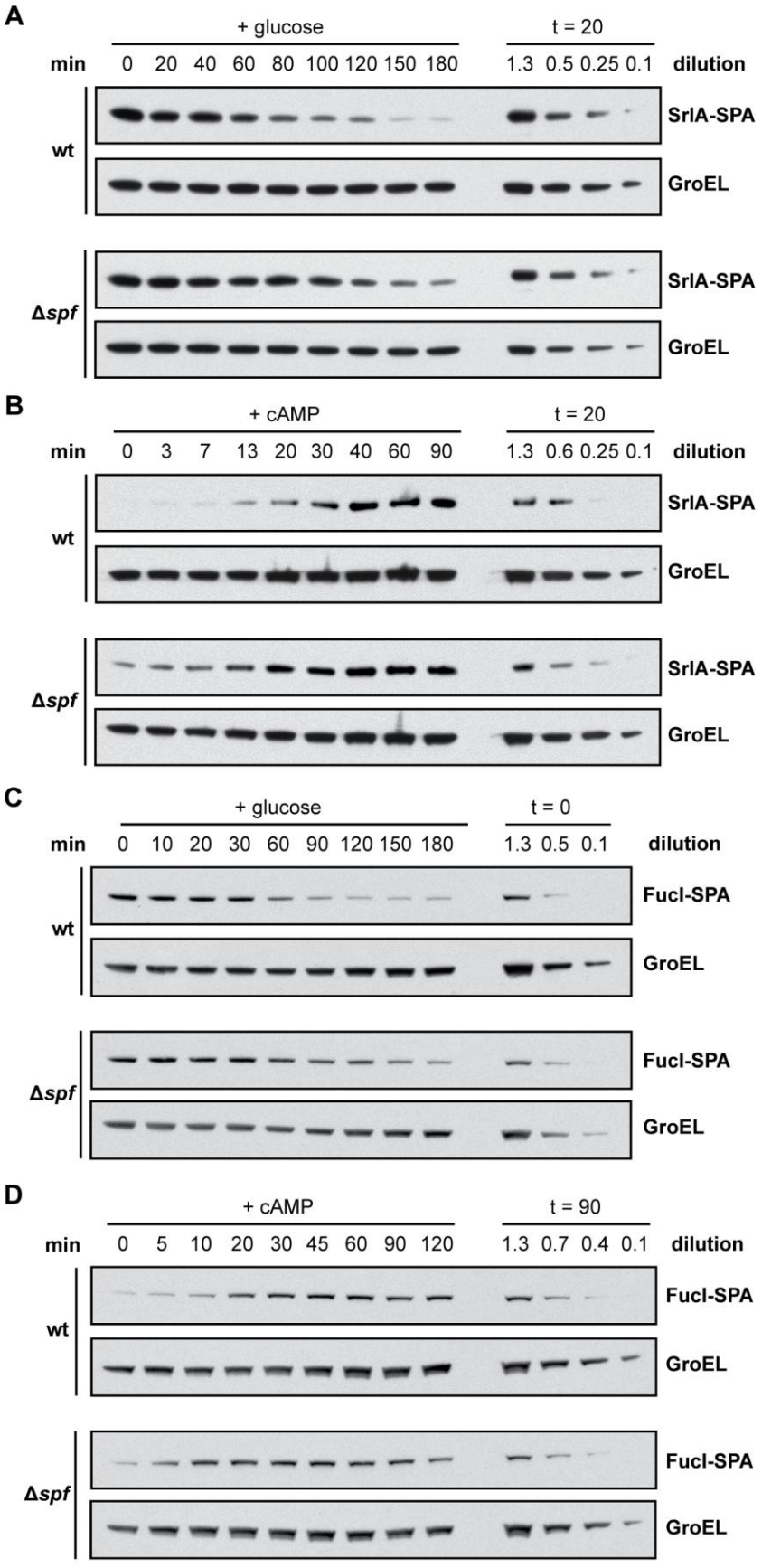

A
wt
Δspf
Δspf Δcrp
wt, - glucose
glucose – + – + – + 1.3 0.6 0.25 0.1 0.05 dilution
SrlA-SPA
GroEL
B
wt
Δspf
Δspf Δcrp
wt, - glucose
glucose – + – + – + 1.3 0.7 0.3 0.1 dilution
FucI-SPA
GroEL
C
wt
Δspf
glucose – + – +
SrlA-SPA
GroEL
Normalized protein levels (AU)
120
100
80
60
40
20
0
wt
Δspf
D
wt
Δspf
– + – + glucose
FucI-SPA
GroEL
Normalized protein levels (AU)
- glucose
+ glucose
wt
Δspf
E
Miller units
1000
100
10
1
wt
Δspf
F
Miller units
1000
100
10
- glucose
+ glucose
wt
Δspf
G
wt
Δspf
Δspf Δcrp
glucose – + – + – +
SrlA-SPA*
GroEL
Spot 42
5S
Normalized protein levels (AU)
120
100
80
60
40
20
0
- glucose
+ glucose
wt
Δspf
Δspf Δcrp

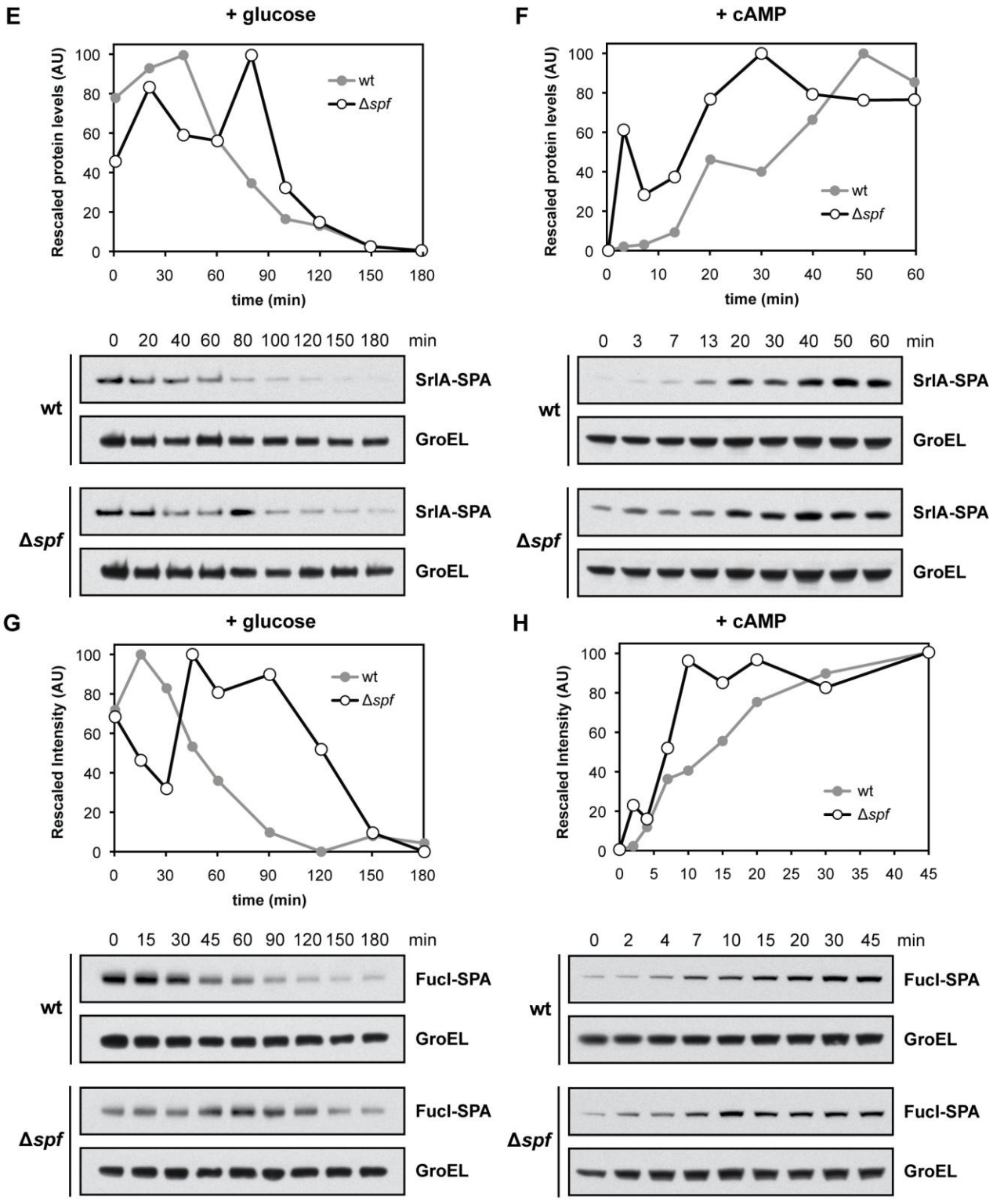
Figure S3. Related to Figure 5

Dilution series for western blot analysis of steady-state measurements and steady-state measurements for strains containing SPA and *lacZ* fusions as well as a destabilized version of SrlA-SPA.

(A–F) (A) and (B) correspond to Figure 5B and Figure 5C, respectively. A dilution series of the wt strain grown in the absence of glucose was included in each gel to generate a standard curve. The standard curve then was used to calculate relative protein levels from background-subtracted intensity measurements followed by normalization to the relative protein levels of the loading control GroEL. Experimental details and notations are as described in Figure 5. Results from quantitative western blot analysis for the wt and Δ*spf* strains of (C) *srlA-SPA* or (D) *fucI-SPA*. Strains were grown in casamino acid-enriched M9 containing 0.4% glycerol or casamino acid-enriched M9 containing 0.4% glycerol and 0.2% glucose. GroEL served as a loading control. Results are representative of two independent experiments. β-galactosidase assay results for wt or Δ*spf* cells containing (E) *srlA-lacZ* or (F) *fucI-lacZ*. Strains were grown in LB or LB containing 0.2% glucose to an OD_{600} of 0.4 – 0.6 and subjected to the β-galactosidase assay. The reported averages and standard deviations for the β-galactosidase assay results are from measurements of cultures from three separate colonies.

(G) Steady-state results from quantitative western blot analysis for wt and Δ*spf* strains containing a destabilized version of SrlA-SPA (denoted SrlA-SPA*). Strains were grown in LB or LB containing 0.2% glucose. See Figure 5B, C for experimental details and notations. The SrlA-SPA fusion was destabilized by introducing a frameshift mutation into the downstream *srlE* gene (*srlE'*). SrlA interacts with SrlE and SrlB to form the IIB and IIC components of the PTS sorbitol transporter in *E. coli*. Disruption of SrlE synthesis destabilizes SrlA by preventing formation of the complete transporter. Results for G are representative of two independent experiments.


A
+ glucose
t = 20
min
0 20 40 60 80 100 120 150 180
1.3 0.5 0.25 0.1
dilution
wt
SrlA-SPA
GroEL
Δspf
SrlA-SPA
GroEL
B
+ cAMP
t = 20
min
0 3 7 13 20 30 40 60 90
1.3 0.6 0.25 0.1
dilution
wt
SrlA-SPA
GroEL
Δspf
SrlA-SPA
GroEL
C
+ glucose
t = 0
min
0 10 20 30 60 90 120 150 180
1.3 0.5 0.1
dilution
wt
FucI-SPA
GroEL
Δspf
FucI-SPA
GroEL
D
+ cAMP
t = 90
min
0 5 10 20 30 45 60 90 120
1.3 0.7 0.4 0.1
dilution
wt
FucI-SPA
GroEL
Δspf
FucI-SPA
GroEL


E
+ glucose
Rescaled protein levels (AU)
time (min)
wt
Δspf
0 20 40 60 80 100 120 150 180 min
SrlA-SPA
GroEL
wt
Δspf
F
+ cAMP
0 3 7 13 20 30 40 50 60 min
G
+ glucose
Rescaled Intensity (AU)
0 15 30 45 60 90 120 150 180 min
FucI-SPA
H
+ cAMP
0 2 4 7 10 15 20 30 45 min

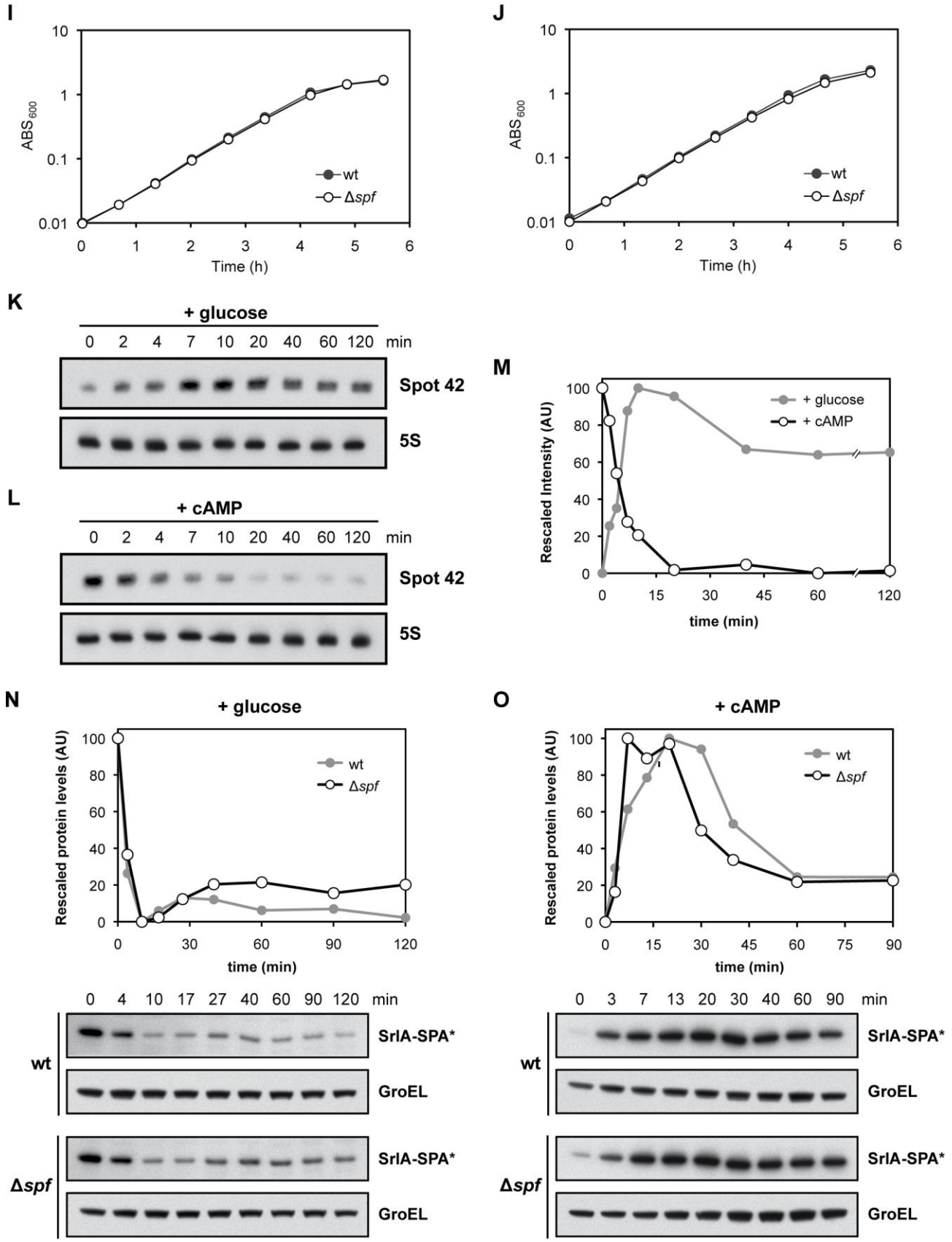

I
J
ABS600
Time (h)
wt
Δspf
K
+ glucose
0 2 4 7 10 20 40 60 120 min
Spot 42
5S
L
+ cAMP
M
Rescaled Intensity (AU)
time (min)
N
+ glucose
O
+ cAMP
Rescaled protein levels (AU)
SrlA-SPA*
GroEL

Figure S4. Related to Figure 6

Dilution series for western blot analysis of time course measurements, independent time course experiments for the *srlA-SPA* and *fucI-SPA* fusion strains, growth rates for strains containing *srlA-SPA* or *fucI-SPA*, northern blot analysis for the activation or repression of Spot 42 expression, and time course measurements for a destabilized version of SrlA-SPA.

(A–D) correspond to Figure 6A–6D, respectively. A dilution series of the indicated time point was included in each gel to generate a standard curve. The standard curve then was used to calculate relative protein levels. The relative protein levels were normalized to the relative protein levels of the loading control GroEL. Experimental details and notations are as described in Figure 6.

(E–H) Results from an independent time course experiment for the *srlA-SPA* and *fucI-SPA* fusion strains. Experimental details and notations are as described in Figure 6.

(I–J) Growth curves for (I) *srlA-SPA* and (J) *fucI-SPA* strains containing the full loop (wt) or the loop reduced to direct regulation by CRP (Δ*spf*). Strains were grown overnight in casamino acid-enriched M9 containing 0.4% glycerol and diluted to an OD_{600} of 0.01 into casamino acid-enriched M9 containing 0.4% glycerol and 0.2% glucose to replicate the experimental conditions of Figure 6A,C. OD_{600} was measured at different times during cell growth. The reported averages are from measurements of cultures from three separate colonies. Error bars representing the standard deviation are smaller than the symbols.

(K–L) Northern blot analysis for the (K) activation or (L) repression of Spot 42 expression. NM525 cells were grown as described in Figure 6A or Figure 6B to activate or repress Spot 42 expression, respectively. 5S RNA served as a loading control.

(M) Quantification of Northern blot analysis results in K and L. Levels were quantified using ImageJ, normalized to the quantified levels of the loading control 5S RNA, and rescaled to span 0 and 100. The reduction in Spot 42 levels ~20 min after glucose addition coincides with the apparent reactivation of CRP (see N) (Pastan and Adhya, 1976).

(N) Time course for SrlA-SPA* levels following deactivation of CRP. Experimental details and notations are as described in Figure 6A. See Figure S3G for a description of SrlA-SPA*.

(O) Time course for SrlA-SPA* levels following activation of CRP. Experimental details and notations are as described in Figure 6B. Results for N and O are representative of three independent experiments.

Supplemental Experimental Procedures

Plasmid construction

The plasmids and the oligonucleotides used in this study are listed in Table S3. Spot 42 variant I was amplified from the genome of NM525 using spf-I.fwd and spf.rev, digested with AatII/EcoRI (New England Biolabs, Ipswich, MA), and cloned into the corresponding restriction sites of pBRplac (Guillier and Gottesman, 2006). Variant II was generated using pSpot42, oligonucleotides spf-II_QC.fwd and spf-II_QC.rev, and the quikchange kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions. Variant III was generated by assembly PCR from the genome of NM525 initially using spf.fwd/spf-III.rev and spf-III.fwd/spf.rev followed by a second PCR using spf.fwd/spf.rev and the products of the initial PCR as DNA templates. All PCR reactions were performed with Platinum *Taq* DNA polymerase high fidelity (Invitrogen, Carlsbad, CA). All constructs were confirmed by sequencing.

Bacterial strain construction

The bacterial strains used in this study are all derivatives of *E. coli* K-12 MG1655 and are listed in Table S3. NM525, the primary strain used in this study, is MG1655 containing the $lacI^q$ mutation. To generate $\Delta spf::kan^R$, the kan^R cassette from pKD13 was amplified with primers spf-rec.fwd and spf-rec.rev by PCR and recombined into the region spanning 37 nts upstream and 68 nts downstream of the *spf* transcription start site using λ red-mediated recombination promoted by pKD46 (Datsenko and Wanner, 2000). The $\Delta spf::kan^R$ allele was moved to a fresh NM525 background and other strains by P1 transduction.

The *lacZ* translational fusion strains were generated using PM1205 as described previously (Mandin and Gottesman, 2009). Briefly, template DNA containing the region spanning the upstream sequence of each gene to the first 9 – 15 codons was inserted in place of the *cat-sacB* cassette located between the pBAD promoter and *lacZ* using mini-λ-mediated recombination (Court et al., 2003). For singly-encoded genes or genes located at the beginning of operons, the mapped transcriptional start site (*gltA*, *nanC*, *srlA*, *xylF*; Condemine et al., 2005; Song and Park, 1997; Wilde and Guest, 1986; Yamada and Saier, 1988) or transcriptional start site determined by 5' RACE (*srlA*, *sthA*) was selected as the 5' end of the mRNA. For genes located in the middle of operons (*fucI*, *fucK*), ~100 nts into the upstream gene was selected as the 5' end for this region. Recombinant strains were selected by growth on sucrose.

To generate GS0446 and GS0447, the kan^R cassette was amplified from pKD13 using either primers fucI_kanR.fwd and fucI_kanR.rev or primers xylF_kanR.fwd and xylF_kanR.rev by PCR and recombined into the ribosome binding site and coding region of *fucI* or *xylF* in NM525 by mini-λ-mediated recombination. The kan^R cassette then was transduced into fresh NM525 by P1 transduction and the kan^R cassette was excised using pCP20.

GS0448, GS0450, and GS0452 were generated by amplifying the SPA tag and the kan^R cassette flanked by FRT sites from pJL148 by PCR (Zeghouf et al., 2004). PCR products were inserted into the 3' end of the chromosomal copy of *srlA* and *fucI* using mini-λ-mediated recombination. The SPA tag and the kan^R cassette then were moved into fresh NM525 by P1 transduction and the kan^R resistance cassette was excised using pCP20 (Cherepanov and Wackernagel, 1995), thereby generating GS0446 and GS0450.

GS0454 and GS0456 were generated by amplifying the region encompassing the promoter of *srlA* to the 3' end of *srlA* or the promoter of *fucP* to the 3' end of *fucI*, respectively,

from NM525 by PCR. PCR products were inserted in place of the pBAD promoter and the *cat-sacB* cassette in PM1205 using mini-λ-mediated recombination. Recombinant strains were selected by growth on sucrose. Recombinations were confirmed by sequencing and transductions were confirmed by PCR.

Growth conditions

All strains were grown by shaking at 250 RPM at 37°C unless otherwise noted. Strains were grown in different media types: Luria-Bertani media containing 1% bacto-tryptone, 0.5% yeast extract, and 1% NaCl (LB); 1X M9 salts supplemented with 10 μg/ml thiamine, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.2% casamino acids (M9). Cell density was obtained by measuring the OD_{600} using an Ultrospec 3000 UV/Vis spectrophotometer (GE Healthcare, Piscataway, NJ).

RNA isolation

Total RNA was isolated using the hot phenol extraction procedure (Aiba et al., 1981). Briefly, pelleted cells were resuspended in 600 μl of 0.5% sodium dodecyl sulfate (SDS), 20 mM sodium acetate, and 10 mM EDTA and mixed with 500 μl of acid phenol:chloroform (Ambion, Austin, TX) pre-incubated at 65°C. After incubating the samples at 65°C for 10 min, the aqueous layer was transferred to a fresh tube. The extraction was performed two more times with acid phenol:chloroform followed by two extraction steps with phenol:chloroform:isoamyl alcohol (Ambion, Austin, TX). The RNA was precipitated with ethanol and resuspended in water. The concentration of RNA following ethanol precipitation was determined using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

Microarray analysis

NM525 cells harboring pBRplac or pSpot42 were grown in LB to an OD_{600} of ~0.4 and treated with 1 mM IPTG. After 7 min, total RNA was isolated as described above and treated with DNase I (New England Biolabs, Ipswich, MA) at 37°C for 30 min. DNase-I treatment was followed by phenol:chloroform extraction and ethanol/sodium acetate precipitation. cDNA preparation and hybridization to the Genechip *E. coli* Genome 2.0 array (Affymetrix, Santa Clara, CA) was conducted as described in the Affymetrix manual Section 3: Prokaryotic Sample and Array Processing (http://media.affymetrix.com:80/support/downloads/manuals/expression_s3_manual.pdf). The complete microarray data set from the three independent experiments was deposited in NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE24875).

Northern blot analysis

Total RNA (5 μg) was resolved either on a 1% agarose gel in 1X MOPS and transferred to a Zeta-Probe GT membrane (Bio-Rad, Hercules, CA) by capillary action for 4 h in 5X SSC and 10 mM NaOH, or on an 8% polyacrylamide gel and transferred to a Zeta-Probe GT membrane using a Trans-Blot (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. In both cases, transferred RNA was cross-linked to the membrane with 120 mJ/cm^2 UV exposure using an HL-2000 HybriLinker (UVP, Upland, CA). Membranes were hybridized in ULTRAhyb hybridization buffer (Ambion, Austin, TX) with ^{32}P-radiolabeled oligonucleotide probes specific to each gene, washed with 0.2X SSC, 0.1% SDS, and exposed to film. The RNA Millenium

Marker (Ambion, Austin, TX) was used as a size marker on the agarose gels and the Perfect Mark RNA Marker (EMD Chemicals, Gibbstown, NJ) was used as a size marker on the polyacrylamide gels.

5' RACE analysis

5' RACE was performed on *srlA* and *sthA* generally as described previously (Mandin and Gottesman, 2009). Total RNA (20 μg) isolated from NM525 was treated with 20 U tobacco acid pyrophosphatase (Epicentre, Madison, WI) at 37°C for 1 h to convert 5' triphosphates to monophosphates. This was followed by treatment with DNase I at 37°C for 15 min to remove DNA. After phenol:chloroform extraction and ethanol/sodium acetate precipitation of the RNA, the 5' monophosphate was removed by incubating the resuspended RNA with 300 U bacterial alkaline phosphatase (Invitrogen, Carlsbad, CA) at 65°C for 1 h. The 5' end of the dephosphorylated RNA was rephosphorylated with 20 U polynucleotide kinase (New England Biolabs, Ipswich, MA) and 2 mM ATP at 37°C for 1 h. This was followed by a second DNase-I treatment at 37°C for 15 min, phenol:chloroform extraction, and ethanol/sodium acetate precipitation. The RNA adaptor PBAD-RNA (50 pmol) was then ligated to the resuspended RNA using 50 U T4 RNA ligase (New England Biolabs, Ipswich, MA) at 16°C for 16 h. The samples were extracted with phenol:chloroform and precipitated with ethanol/sodium acetate. The resuspended RNA was reverse transcribed with 15 U Thermoscript reverse transcriptase (Invitrogen, Carlsbad, CA) using oligonucleotide srlA_RT1 or sthA_RT1 according to the manufacturer's instructions. The 5' end of *srlA* and *sthA* was then amplified by PCR using oligonucleotides PBAD-DNA and either srlA_RT2 or sthA_RT2, respectively. The amplified

cDNA fragments were directly transformed into PM1205. The 5' end was mapped by sequencing at least three colonies.

β-galactosidase assays

Three colonies from each transformed strain were inoculated into separate tubes containing LB and grown overnight. Overnight cultures were diluted into 3 ml LB to an OD_{600} of 0.01 and grown to an OD_{600} of ~0.1. Either 0.2% arabinose or 0.2% arabinose together with 1 mM IPTG was added to each culture. After 1 h, the OD_{600} of the cells was measured and 100 µl of cells were lysed in 700 µl Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, and 3.4% β-mercaptoethanol) with 15 µl 0.1% SDS and 30 µl chloroform. The β-galactosidase assays were performed as described previously (Miller, 1972).

To assay the *srlA-lacZ* and *fucI-lacZ* strains containing full-length *srlA* and *fucI*, three colonies of each strain were grown overnight in LB or LB + 0.2% glucose. Overnight cultures were diluted into 3 ml of the same media to an OD_{600} of 0.01 and grown to an OD_{600} of 0.4 – 0.6. Cultures then were harvested and assayed for β-galactosidase activity as described above.

Base pairing prediction

The location of base pairing between Spot 42 and different genes was predicted using the folding algorithm *NUPACK* (http://nupack.org/). The portion of each *lacZ* fusion corresponding to the integrated gene was folded with the entire Spot 42 sequence. Parameters values used in all folding predictions were 10 µM Spot 42, 1 µM target mRNA, and a temperature of 37°C. Regions of base pairing are reported in Figure 3C. *NUPACK* can account for both intramolecular and intermolecular base-pairing interactions between multiple nucleic acid strands. Thus, this

algorithm penalizes against intramolecular base pairs that break up secondary structures in each RNA molecule.

Quantitative western blot analysis

For the steady-state measurements, overnight cultures were diluted to an OD_{600} of 0.01 in the same type of media and grown until an OD_{600} of 0.4 – 0.6. For the dynamic measurements, overnight cultures were diluted to an OD_{600} of 0.01 into 9 or 10 tubes containing the same type of media and grown to an OD_{600} of 0.4 – 0.6. At the indicated time prior to harvesting the cells, 0.2% glucose (CRP deactivated) or 10 mM cyclic AMP (CRP activated) was added to each tube.

To harvest the cells, 1 ml of each culture was mixed with 0.4 ml ice-cold 1X PBS, and cells were pelleted and frozen on dry ice. Frozen cells were resuspended in 0.25X stacking buffer (Biofluids), 0.5% SDS, 12.5% glycerol, 5% β-mercaptoethanol to yield an equal concentration of cells in each sample. 10 µl of each sample was resolved on a Novex 15-well 16% tricine gel (Invitrogen, Carlsbad, CA) using the XCell *SureLock* Mini-Cell (Invitrogen, Carlsbad, CA). Resolved proteins were transferred to a 0.45 µm pore nitrocellulose membrane (Invitrogen, Carlsbad, CA) in 1X Novex Tris-glycine transfer buffer (Invitrogen, Carlsbad, CA), 0.2% methanol, and 0.4% SDS using a Mini Trans-Blot Cell (Bio-Rad, Hercules, CA). The SPA tag was detected using the mouse monoclonal ANTI-FLAG M2-alkaline phosphatase antibody (1:2,000 dilution, Sigma) with the Lumi-Phos WB reagent (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. The loading control GroEL was detected with the mouse anti-GroEL monoclonal antibody (1:20,000 dilution, Abcam) and the Immunopure goat anti-mouse IgG-horseradish peroxidase (1:40,000 dilution, Pierce) with SuperSignal West Pico chemiluminescent substrate (Pierce) according to the manufacturer's instructions.

Protein levels were calculated using the ImageJ software package (Rasband, 1997-2009). Background intensity measured directly below each band was subtracted and a dilution series in the same gel was used to calculate relative protein levels. For each time point, relative protein levels of the SPA fusion were divided by relative protein levels of the loading control GroEL to yield normalized protein levels. For the dynamic measurements, normalized protein levels were rescaled to span 0 to 100 in order to directly compare the dynamics between strains.

Supplemental References

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